Type: Poster Presentation

Final Abstract Number: 64.009
Session: Virology and Viral Infections (Non-HIV) II
Date: Saturday, April 5, 2014
Time: 12:45-14:15
Room: Ballroom

Role of the rabies virus phosphoprotein in the induction of mitochondrial dysfunction in rabies virus infection

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Background: Previous studies in an experimental model of rabies showed neuronal process degeneration in association with severe clinical disease. Cultured adult rat dorsal root ganglion (DRG) neurons infected with CVS-11 strain of rabies virus (RABV) showed axonal swellings and immunostaining for 4-hydroxy-2-nonenal (4-HNE) indicating evidence of lipid peroxidation associated with oxidative stress and reduced axonal growth versus mock-infection. We have demonstrated that RABV induces alterations in a variety of mitochondrial parameters, including an increase in reactive oxygen species production after addition of substrates or inhibitors and increased activity of Complex I of the respiratory chain vs. mock infection. We have hypothesized that a RABV protein targets the mitochondria and triggers its dysfunction.

Methods & Materials: We used immunocytochemistry, Western immunoblotting, immunoprecipation and proteomics methods to study RABV- and mock-infection of mouse neuroblastoma (MNA) cells.

Results: Immunocytochemistry of CVS-infected cells after 72 h post-infection showed strong colocalization of mitochondria with the RABV phosphoprotein (P). Extracts of MNA cells from the mitochondrial subcellular fractionation were analyzed with a proteomics approach. We identified peptides belonging to the RABV P, glycoprotein (G), and nucleocapsid protein (N) with 67% of protein coverage at 95% confidence for P vs. N (8%) and G (6%). Our data show that the majority of viral peptides (79%) belong to P vs. N (13%) or G (8%), indicating that the extract was highly enriched with P. P was detected by immunoblotting in RABV-infected purified mitochondrial extracts and in Complex I immunoprecipitates from the extracts, but not in mock-infected extracts. A plasmid expressing P in cells produced an increase in Complex I activity, whereas expression of other RABV proteins did not. We have analyzed a variety of recombinant plasmids encoding various segments of the P gene. Expression of a peptide from amino acid 139 to 172 produced an increase in Complex I activity similar to expression of the entire P protein, whereas most peptides that did not contain this region did not produce increased activity of Complex I.

Conclusion: The RABV phosphoprotein is present in mitochondria and may interact with Complex I causing mitochondrial dysfunction, oxidative stress, neuronal process degeneration, and severe clinical disease in experimental rabies.

http://dx.doi.org/10.1016/j.ijid.2014.03.1339

Type: Poster Presentation

Final Abstract Number: 64.010
Session: Virology and Viral Infections (Non-HIV) II
Date: Saturday, April 5, 2014
Time: 12:45-14:15
Room: Ballroom

Association of toll-like receptor 3 polymorphism with chronic hepatitis B

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Background: The immunopathogenesis of chronic hepatitis B has not been clarified yet. Toll like receptors (TLR) are a receptor family that initiates immunity with exogen-endogen ligands and play role in the pathogenesis of hepatitis B infection. In this study we aimed to investigate the association of TLR3 polymorphism with chronic hepatitis B patients.

Methods & Materials: The total 73 active and 43 inactive hepatitis B patients and 50 healthy individuals as control group were included to the study between 01 March 2013 and 31 August 2013. Genomic DNA was extracted from whole blood samples and single nucleotide polymorphism (1377 C/T) were studied. Restriction fragment length polymorphism (RFLP) method was used to determine TLR 3 gene (1377 C/T) polymorphism in PCR samples.

Results: Statistical difference was determined in CC, CT, TT genotypes and HBV DNA levels in chronic hepatitis B patients ($p = 0.013$). The highest levels of HBV DNA were detected in individuals with TT genotypes. Data showed that subjects carrying CC and TT genotype had 2.4 fold increased risk of chronic HBV infection compared to those with CT genotype (95% confidence interval (CI) = 1.04-5.5, and $p = 0.037$).

Conclusion: In our study HBV DNA was higher in individuals with TT genotypes, and CC and TT genotypes were more commen in active chronic hepatitis B patients. These results indicate that TLR3 1377CT polymorphism could be a protector factor for the development of chronic HBV infection. Further studies are needed to clarify the relation between TLR 3 gene polymorphism and chronic hepatitis B.

http://dx.doi.org/10.1016/j.ijid.2014.03.1340